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PUBLIC HEALTH PROBLEMS IN CIVIL DEFENSE. METHODS FOR THE DETECTION AND IDENTIFICATION OF CHEMICAL WARFARE AGENTS IN AQUEOUS CARRIERS

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PUBLIC HEALTH PROBLEMS IN CIVIL DEFENSE

Methods for the Detection and Identification of Chemical Warfare Agents in Aqueous Carriers

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SUMMARY

1. Among the chemical warfare agents that might be encountered in civil defense are sarin, tabun, and soman (G-agents); sulfur and nitrogen mustards; arsenicals, cyanides, and fluoro-organic compounds.

2. In this discussion six rapid colorimetric tests are recommended for the quantitative determination of these agents in water. The tests are adaptable to both laboratory and field conditions.

3. A general review is presented of methods for the detection and quantitative determination of certain chemical warfare agents which might be encountered in civil defense.

4. Most of the methods described in existing literature lack specificity.

INTRODUCTION

AGENTS

The development of newer and more toxic warfare agents and the more rapid means of disseminating them over greater distances have increased the need for civilian authorities to be able to detect these toxic warfare agents in air, water, and food. Defense against them may often depend on early detection. Identification is necessary not only for prevention of casualties, but also to determine the medical treatment and methods of decontamination needed. With the development of more toxic agents, those likely to be used have been reduced to a relatively small group. Of the newer agents, the most dangerous are those referred to as the "G-agents" or nerve gases, organophosphate compounds which violently interfere with the nervous system. Other important agents are the nitrogen and sulfur mustards and, to a lesser extent, a few of the arsenicals and cyanide agents. This review is limited to chemical methods for detection and determination of these four types of agents.

The four groups of chemical warfare agents important to civil defense are tabulated below:

Group	Symbol	Name	Formula	Action
G-Series	GA	Tabun	$CN(CH_3)_2NPOC_2H_5O$	Cholinesterase inhibitor
	GB	Sarin	$F(CH_3)_2CHOPOCH_3$	"
	GD	Soman	$FCH_3OPO(CH_3)_3CCHCH$	"
Mustards	HD	Sulfur mustard	$(ClCH_2CH_2)_2S$	Vesicant
	HN-1	Nitrogen mustard	$(ClCH_2CH_2)_2NC_2H_5$	"
	HN-2	Nitrogen mustard	$(ClCH_2CH_2)_2NCH_3$	"
	HN-3	Nitrogen mustard	$(ClCH_2CH_2)_3N$	"
	L	Lewisite	$ClCH{:}CHAsCl_2$	Vesicant
	ED	Ethyldichloroarsine	$C_2H_5AsCl_2$	"
	AC	Hydrogen cyanide	HCN	Systematic
	CK	Cyanogen chloride	CNCl	"

RAPID TESTS

General methods for detection of each group are of value since they cover a wider range of possible contaminants and limit the number of tests necessary to identify any particular agent. The presence of interfering substances which might result in a false positive or a negative result varies with the type of sample. Methods designed for the detection of agents in air are generally more accurate than for those in water, because there are less interfering substances in air.

Most of the methods are based on the color change of a reagent or of silica gel or test paper impregnated with the reagent, depending upon whether the contaminant is in water, air, or deposited on surfaces. Taken separately, however, they are seldom specific. Such tests are generally simple, sensitive, and rapid, and their value greatly depends on the circumstances under which the tests are made and the intelligence used in interpreting the results.

There are six recommended tests for the detection or quantitative determination of the most dangerous chemical warfare agents in water. These tests will produce reliable results if the water was of fair quality before it became contaminated with the agent.

Chlorine Demand Test

The value of this test for the detection of chemical warfare agents depends on the demand of the water before contamination. With treated water having little or no demand, a chlorine demand may indicate the presence of tabun, sulfur mustard, lewisite, or hydrogen cyanide. In conjunction with other tests chlorine demand can be employed to differentiate between the presence of sulfur and nitrogen mustards, cyanogen chloride and hydrogen cyanide, and between tabun and sarin or soman.

Test for G-Agents

Tabun, sarin, and soman can be detected by oxidation with alkaline peroxide to form an intermediate product capable of oxidizing o-tolidine in an alkaline solution to form a yellow dye. By extracting the color with an organic solvent, the sensitivity can be increased and results can be interpreted quantitatively to as low as 0.1 ppm. Reducing agents or water with a chlorine demand greater than 10 ppm will produce negative or low results. Such interferences can be eliminated by first satisfying the chlorine demand of the sample. Several organophosphate insecticides, which are currently used and only slightly less toxic than the G-agents, will also produce positive tests. Thus, for all practical purposes they may be considered G-agents.

Test for Mustard Agents

Sulfur and nitrogen mustard (HN-1, HN-2 and HN-3) form additional compounds when heated with gamma-(4-nitrobenzyl) pyridine, producing an intense purple-blue color when treated with alkali. By comparison with standards, quantitative results can be obtained from samples containing as low as 5 ppm of the mustards. Intermediate hydrolyzed products of the agents produce shades of color that are distinctively bluer than those produced by the unhydrolyzed agent. This can interfere with accurate results but an estimate of mustard content can be made.

Test for Arsenicals

All the arsenicals can be detected or determined quantitatively by oxidation and conversion of the organic arsenical to an arsenate ion. This ion can then be determined by the molybdenum blue or the Gutzeit method. The results in terms of arsenic can be interpreted as ppm of lewisite. The Gutzeit method is not affected by any interferences, but it is difficult to interpret the results quantitatively. The molybdenum blue method will produce high results if silicates or phosphates are present in the water. However, the presence of these interferences can be determined by omitting the oxidizing agents.

Test for Cyanides

The presence of cyanides can readily be detected by converting cyanogen chloride which produces a yellow complex with gamma (4-nitrobenzyl) pyridine. In the range of 0.5 to 100 ppm cyanide, the test is accurate for the determination of hydrogen cyanide, cyanogen chloride, and the cyanide hydrolyzed from tabun. When the chlorine demand of the contaminated water exceeds 10 ppm, low values might result. This can be eliminated by first reducing the chlorine demand. Cyanogen chloride can be determined separately by direct addition of gamma-(4-nitrobenzyl) pyridine.

pH Determination

Almost all chemical warfare agents hydrolyze to some degree in water giving an acid hydrolysis product. The degree of hydrolysis is dependent upon the agent, temperature, pH, and time. Water having pH 6 or lower should be strongly suspected of contamination and should not be used until confirmed otherwise.

RECOMMENDED PROCEDURES

I. RAPID CHLORINE DEMAND TEST

A. Reagents

1. Sulfuric acid or powdered potassium bisulfate.
2. Chloramine T; 5% aqueous solution.
3. Potassium iodide; 75% aqueous solution.
4. Sodium thiosulfate; 0.0563 N, 1 ml is equivalent to 2 mg Cl_2.
5. Starch solution.
6. Sulfamic-potassium bisulfate reagent; dissolve 2 g of sulfamic acid in 100 ml of 8.2% potassium bisulfate solution.

B. Standardization of chloramine T solution

1. To 100 ml of distilled water add 8.2 g of potassium bisulfate or sufficient sulfuric acid to reduce pH to 1.0 to 2.0.
2. Add exactly 1.0 ml of 5% chloramine T. Mix and allow to stand 5 minutes.
3. Add 1 ml of potassium iodide reagent and titrate against 0.0563 N thiosulfate from burette to a pale yellow color.
4. Add 1 ml of starch and continue titration until disappearance of blue color.
5. Record ml of thiosulfate added as titer A.

C. Chlorine demand of contaminated water

1. To 100 ml of water sample add 1 ml of 2% sulfamic acid in 8.2% potassium bisulfate. Mix and allow to stand 10 minutes.
2. Lower pH to about 1.0-2.0 by addition of 8.2 g of potassium bisulfate or concentrated sulfuric acid.
3. Continue procedure as in the standardization of the chloramine T solution, starting with the addition of 1 ml of chloramine T.
4. If no chlorine remains at the end of the 5 minute contact time, repeat the test using 2.0 ml (or more if necessary) of the chloramine T solution.
5. Record the amount of thiosulfate used in titrating the residual chlorine as titer B.
6. Calculations:
   ppm chlorine demand = 20 [(ml chloramine T added x titer A) - titer B]

D. Interpretation of results

Positive chlorine demand tests in raw water may be due to many natural agents in water, such as hydrogen sulfide and organic or bacterial contamination. In addition it may also indicate the presence of sulfur mustard and its hydrolytic product, lewisite, methlydichloroarsine, ethyldichloroarsine, diphenylcyanoarsine, and free cyanide from hydrogen cyanide and tabun. Nitrogen mustards, sarin and soman or their hydrolytic products do not exert a chlorine demand at a low pH. In treated water (finished) having no chlorine demand immediately prior to contamination, sulfur mustard or lewisite exerts a chlorine demand that is equivalent to the amount of agent added.

II. DETERMINATION OF G-AGENTS IN WATER

A. Reagents

1. Acetone; ACS specifications.
2. Ortho-tolidine dihydrochlide; 1% in water.
3. Sodium perborate; 1% aqueous solution (Dupont's Perdox, low ratio alkalinity is recommended), prepare fresh daily.
4. Disodium phosphate; 0.5 M aqueous solution.
5. Standard GB solution, or Hellige color comparator disc No. 860-14.
6. Xylene; U.S.P.

B. Procedure

1. Determine chlorine demand on 100 ml aliquot of sample. If the demand is more than 10 ppm, reduce the demand of 100 ml by adding a calculated amount of standard chloramine T solution (c.f. Chlorine Demand Determination).
2. To 50 ml of sample (chlorine demand less than 10 ppm) in a 100-ml glass-stoppered graduate, add the following reagents and mix after each. Caution: direct sunlight will produce false-positive results.

   10 ml of acetone
   1 ml of disodium phosphate solution
   2 ml of o-tolidine reagent
   5 ml of sodium perborate solution

3. Add 10 ml of xylene without mixing.
4. Stopper the graduate and allow 10 minutes for color to develop.
5. Extract color from aqueous layer into xylene by inverting the graduate several times.

B. Procedure (cont'd)

6. Allow solvent layer to separate from the aqueous layer.

7. Pipette off the solvent layer and determine the optical density of it by either of the following two methods.

   a. Hellige color comparator disc No. 806-14. Transfer xylene layer to 13 mm. Hellige cell and compare against bank (xylene), using color comparator disc. The disc contains eight permanent glass standards equivalent to 0, 0.20, 0.50, 1.00, 2.5, 5.0, 10.0, 20.0, and 30.0 ppm of GB.

   b. Electrophotometric method. Prepare a series of GB standards containing from 0 to 0.75 mg (15 ppm) in 50 ml distilled water. Develop color in same manner as with the sample. Determine the optical densities of the standards in Klett-Summerson photometer using 1.3 cm cells and a No. 42 filter (420 mu wavelength).

III. DETERMINATION OF MUSTARDS IN WATER

A. Reagents

1. Hydrochloric acid; dilute 1:50.
2. DB-3 reagent; 5% gamma-(4-nitrobenzyl) pyridine in acetone.
3. Acetone; ACS specifications.
4. Potassium carbonate; 0.1 M solution.
5. Standard nitrogen mustard (HN3) solution or Hellige color comparator disc No. 860-13.

B. Procedure

1. Adjust pH of 100 ml of the sample to 4.0 $\pm$ 0.2 by addition of a few drops of dilute HCl; also adjust 100 ml of distilled or deionized water to the same pH.
2. Pipette exactly 4.0 ml of suspected water (or an aliquot diluted to 4.0 ml with distilled water adjusted to pH 4) into a test tube. Sample should contain less than 0.060 mg HN3 or 0.080 mg HD per 4 ml.

B. Procedure (cont'd)

3. Prepare a reagent blank (4 ml distilled adjusted to pH 4) and treat in same manner as sample.
4. To sample and blank add exactly 0.5 ml of DB-3 reagent and mix until cloudiness is evenly distributed. Appearance of a yellow color at this step indicates presence of cyanide.
5. Place the tubes in a boiling water bath for 10 minutes. If the sample is high in hardness, heat for 15 minutes in bath.
6. Cool under tap water.
7. Add 4 ml of acetone and 0.5 ml of 0.1 M potassium carbonate. Mix thoroughly.
8. Determine the density of the color exactly 5 minutes after the addition of the last reagent. The relationship of color density to concentration of agent can be determined by either of the following methods.

a. Hellige color comparator disc No. 860-13. Transfer sample to 13 mm Hellige cell and compare against blank using color comparator disc. This disc was prepared by the Chemical Corps for the above procedure. The disc gives the color densities equivalent for 5, 10, 15, and 20 ppm of HN3 when only a small quantity of the agent is hydrolyzed. The test is positive for H, HN1, HN2, and HN3. Shades of color distinctively bluer than the purple discs are caused by their hydrolyzed products.

b. Electro-photometric method. Prepare a series of HN3 standards containing up to 60 micrograms per 4 ml of distilled water (pH 4). Develop color in same manner as sample. Determine optical densities of the standards and sample in a Klett-Summerson photometer using 13 mm cell and a No. 66 (660 millimicrons) filter. One microgram of HN3 is equivalent to approximately 2.0 Klett units.

IV. DETERMINATION OF ARSENICALS IN WATER

A. Reagents

1. Sodium perborate; 1% aqueous solution, prepare fresh daily (recommended DuPont's "Perdox").
2. Potassium bisulfate solution; 8.2% aqueous.
3. Hydrazine sulfate; 0.6% aqueous solution.
4. Ammonium molybdate; 1% in 4.5 N sulfuric acid.

B. Procedure

1. Pipette 2 ml aliquot of sample into a test tube and dilute to 4.0 ml with distilled water.
2. Prepare a blank of 4.0 ml of distilled water and treat in same manner as sample.
3. To each add 2 drops of potassium bisulfate and 0.5 ml of sodium perborate.
4. Mix and place in boiling water bath for 20 minutes.
5. Add 0.5 ml of hydrazine sulfate and 0.5 ml of ammonium molybdate reagent.
6. Mix and continue heating in water bath for 5 minutes more.
7. Compare color with Hellige disc or in photometer with a series of standards.
   a. Hellige war gas determination disc No. 860-13. Place sample and blank in 13 mm cells in the comparator and compare color with discs. The standards on disc are given as the equivalent lewisite concentration of the arsenic present.
   b. Klett-Summerson photometer. Prepare a series of standards containing 20, 30, 40, and 50 ppm lewisite or their equivalent in arsenic concentration. Mg. lewisite x 0.3613 is equivalent to mg arsenic, or mg arsenic x 2.823 is equivalent to mg lewisite. Develop color in same manner as sample. Read absorbance in photometer using 13 mm cell and No. 66 filter (660 mu wavelength).

C. Interpretation of results

Water samples high in phosphates or silicates may give false positive results. If they are suspected, repeat the test, omitting the addition of sodium perborate reagent. When this reagent is omitted the organic arsenic will not be converted to the proper form to give a color reaction. Any color formed in this control test is attributed to phosphates or silicates. The difference between the two readings represents the true concentration of arsenicals in the sample.

V. DETERMINATION OF CYANIDE OR CYANOGEN CHLORIDE IN WATER

A. Reagents

1. Chloramine T; 5% aqueous solution.
2. Sodium arsenite; 0.455% aqueous solution.
3. Acetone; ACS specifications.
4. DB-3 reagent; 5% gamma-(4-nitrobenzyl) pyridine in acetone.
5. Standard cyanide solution; 0.251 g KCN per liter water, 0.1 mg cyanide per ml, or Hellige color comparator disc No. 860-12.

B. Procedure

1. Determine chlorine demand on 100 ml aliquot of sample. If the demand is more than 10 ppm reduce the demand of 100 ml of the sample by addition of a calculated amount of standard chloramine T solution. (c.f. Chlorine Demand Determination.)
2. Pipette 5.0 ml of the sample (chlorine demand less than 10 ppm) into a 13 mm Klett cell or test tube.
3. Add 0.1 ml of 5% chloramine T solution followed immediately by addition of 1 ml of sodium arsenite.
4. After 1/2 minute add 0.5 ml of DB-3 reagent and 1.5 ml of acetone.
5. Dilute to 10 ml with distilled water and mix.
6. After 5 minutes (and not later than 10 minutes) compare color density with a Hellige standard chlorine disc (No. 860-12) or in a Klett-Summerson photometer with a series of cyanide standards.

a. Hellige comparator with standard chlorine disc.

Place sample in center compartment and blank (sample without reagents) in right-hand compartment. Match color of sample with one on the disc and record results in ppm chlorine.

Reading on chlorine disc	Equivalent ppm cyanide
0.1	0.5
0.15	1.0
0.25	2.0
0.50	5.0
1.0	15.0
1.5	40.0
2.5	60.0
5.0	80.0
10.0	100.0

b. Klett-Summerson photometer.

Prepare a series of cyanide standards containing from 0.0 to .0005 mg of CN^- in 5 ml volumes. Develop color of the standards in same manner as the sample, starting with the addition of 0.1 ml 5% chloramine T. Determine the optical densities of the standards and the sample in the photometer, using 13 mm cells and a No. 42 filter (420 mu wavelength).

C. Interpretation of results

A positive cyanide test indicates the possible contamination with AC, CK, or GA. If CK is suspected from formation of yellow color in the mustard test, the following determination for CK should be made: Repeat the above procedure for cyanides on sample without satisfying the chlorine demand and omit the addition of dichloramine T and of sodium arsenite. The final results are in ppm cyanide due to CK. To convert to ppm CK, multiply the results in ppm cyanide by the factor 2.4.

Recommended Chemical Methods of Identification of C. W. Agents

Agent	Type of Sample	Reagents or Test	Nature of Positive Result	Remarks
G-series	Water Air Food	Alkali peroxide o-tolidine	Yellow color	Reducing agents producing a chlorine demand > 10 ppm interfere.
GA	Water	Cyanide test with DB-3 reagent	Yellow color	Test for the cyanide hydrolytic product of tabun.
GB		No known specific test		
GD		" " " "		
Mustards	Water Air	DB-3 reagent, heat and alkali	Reddish-blue color	Reacts with other chemical compounds that act as strong alkylating agents.
Nitrogen Mustards	Water Air Food	Dragendorff's reagent	Orange-red color	Reacts with nitrogen ring bases and some substituted ammonia compounds.
Sulfur mustard	Water	Chlorine demand test		In water sulfur mustard has a chlorine demand equal to the concentration of the agent. Nitrogen mustards have no chlorine demand.
Arsenicals	Water	Molybdenum blue method	Blue	Test for arsenic ion; phosphates and silicates in water interfere.
	Water	Gutzeit method	Yellow-brown stain on $HgCl_2$ paper	Semi-quantitative results.
Lewisite	Air Food	Cuperous acetylide test	Reddish brown color	
Cyanides	Water Air Food	DB-3 reagent with chloramine-T and sodium arsenite	Yellow color	Test base on the detection of cyanide ion.
Cyanogen Chloride	Water Air	DB-3 reagent without dichloramine-T and sodium arsenite	Yellow to orange color	

Summary of Results of Recommended Tests

Agents	G-agent test	Mustard test	Arsenic test	Cyanide test	Cyanogen test	Chlorine demand	pH	Hydrolysis rate (3)
Tabun	+						< 6	VS
hydrolyzed product (HCN)				+		+	< 6	
Sarin	+						< 6	VS
Soman	+						< 6	VS
Sulfur mustard		+				+	< 6	S
Nitrogen mustards		+					< 6	S
hydrolyzed products		+(1)					< 6	
Lewisite			+(4)			+	< 6	F
hydrolyzed products			+				< 6	
Cyanogen chloride				+	+		< 6	VS
Hydrogen cyanide				+		+	< 6	VS

(1) Intermediate hydrolyzed products produce a bluer shade than the purple produced by the agents.
(2) Silicates and phosphates give false positive results.
(3) Comparative rate at conditions (pH, temperature, and quantity of dissolved solids) of most natural waters; F fast, S slow, VS very slow.

SUPPLEMENTARY DATA

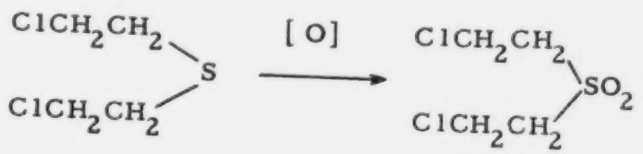
MUSTARD C. W. AGENTS

The mustard group of C. W. agents are divided into two particular classes of compounds; the sulfur mustards (chloroalkylsulfides), and the newer nitrogen mustards (chloroalkylamines). Although many analogues of these may exist, the only four specific compounds considered as chemical warfare agents are listed below.

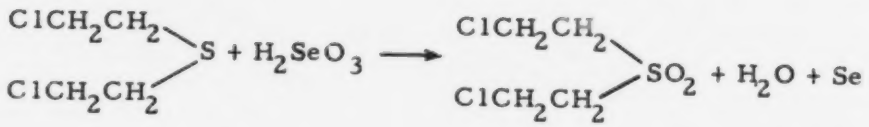
Structure	Chemical Name	Symbol
$(ClCH_2CH_2)_2S$	Bis (2-chloroethyl) sulfide	HD
$(ClCH_2CH_2)NC_2H_5$	Bis (2-chloroethyl) ethylamine	HN-1
$(ClCH_2CH_2)_2NCH_3$	Bis (2-chloroethyl) methylamine	HN-2
$(ClCH_2CH_2)_3N$	Tris (2-chloroethyl) amine	HN-3

The nitrogen mustards have never been used in warfare. Therefore, only a few reliable methods of detection have appeared in literature. However, since the sulfur mustards were used several times during World War I, the need of their detection has been emphasized.

Some of the earlier means of detecting sulfur mustard were based on the oxidation of the sulfide ion:



By passing the vapors through a small quantity of a slightly acidic solution of 0.003% potassium permanganate, Spica[1] observed that a color change was produced by as little as 0.15 mg of dichloroethyl sulfide. Later Yablick[2] established a method based on the same principle, but the oxidizing agent employed had a higher degree of specificity for sulfur mustard. A warm acidic solution of 1% selenium dioxide was reduced to a reddish-orange precipitate of metallic selenium by 5 mg of the vapor. Selenium dioxide, however, is also reduced by hydrogen sulfide, sulfur dioxide, carbon monoxide, and hydroxylamine.



This method was adapted to a semi-quantitative test by measuring the amount of selenium suspension by nephelometric or colorimetric procedures.[3]

Grignard's Reagent [4], which is more specific for bis (2-chloroethyl) sulfide, is based on a double decomposition reaction with hydrogen iodide. In 1 ml of reagent, the formation of diiodo-ethyl sulfide crystals can be detected by 0.025 mg of sulfur mustard vapors in air.

The formation of insoluble additive compounds of mustard with gold or palladium chloride solutions was employed by Schrater[5]. As low as 10 mg of vapor could be detected by the formation of turbidity in a 0.1% gold chloride or 0.05% palladium chloride solution. The same reaction was also carried out on filter paper impreganted with the reagent on which droplets of mustard produce a reddish-brown stain with gold chloride and a yellow stain with palladium chloride. Obermiller[6] modified the procedure slightly by collecting the vapors on silica gel and then applying gold chloride and hydrogen peroxide solutions.

The ability of dichloroethyl sulfide to release free iodine from sodium iodoplatinate has long been recognized. Spica[1] moistened filter paper with 0.2% sodium iodoplatinate solution. A color change from the original pink to violet was produced by 0.02 mg of mustard. The same reaction was applied by Lockwood[7] in testing for agent extracted from food with benzene and titrated with a standard sodium iodoplatinate. By passing the mustard vapors through glass wool moistened with 5% acetic acid and then adding 2 drops of iodoplatinate reagent and 2 drops of starch solution, the sensitivity can be increased to 0.005 mg of sulfur mustard[8]. Chlorine and nitrous fumes will also give a positive test. Recently, Allsopp[9] employed a similar procedure as a semi-quantitative method for sulfur mustard in aqueous solutions. The intensity of the resulting blue color was read quantitatively with an absorptiometer. The results were reproducible to 1-3% over a range of 5-50 ppm of sulfur mustard. However, free chlorine in the water interferes.

The amount of chlorine available from dichloramine T, after part is used in oxidizing the agent, offers a method of determination which

is similar to the chlorine demand test. Kinsey and Grant[10] extracted the sulfur mustard from water with a mixture of 80% cyclohexane and 20% purified kerosene. The extraction removed only the unhydrolyzed agent. A standard amount of dichloroamine T was added to the solvent and the demand determined at specific condition by iodometric titration. With this procedure they were able to determine mustard in the range of 0.5 to 200 micrograms.

In studying some of the reactions of mustard, Rebas et al[11] found that in an aqueous solution of a heterpoly acid and gold chloride, sulfur mustard formed a milky turbidity due to the formation of a Werner coordination complex. The author states that this reaction was specific and sensitive to 5 ppm of mustard. Ruchhoft and Placak[12] investigated the conditions for greatest sensitivity of sulfur mustard with phosphotungstic acid containing gold chloride, and considered its usefulness as a field test for sulfur mustard in water. They determined that positive tests could be obtained from 0.06 mg of mustard at low concentration (5 ppm), or as little as 0.003 mg at very high concentrations (90 ppm). The sensitivity is not affected by low salt concentration found in most natural waters or by up to 70 ppm of thiodiglycol. This test can be employed as a semi-quantitative method by varying the quantity of sample and reagents to produce an opalescence or slight turbidity within 2 to 5 minutes. Nitrogen mustard also gave positive tests; therefore, to differentiate between nitrogen and sulfur mustard, the authors recommended application of the iodoplatinate test for sulfur mustards after positive results are obtained with the phosphotungstic acid - gold chloride test.

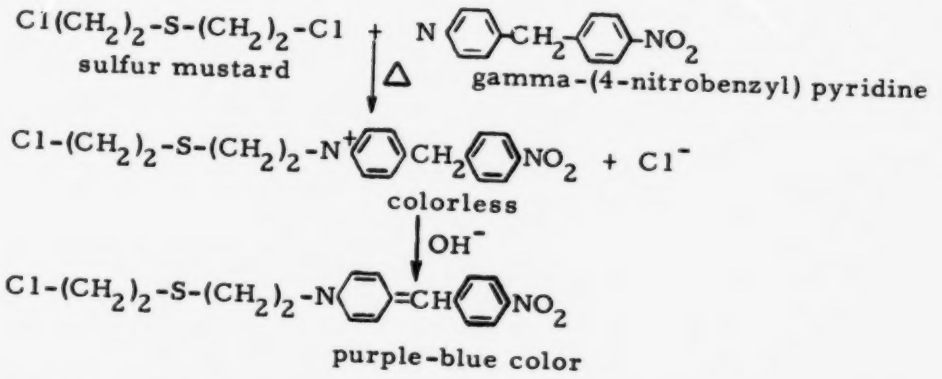
The Army Chemical Corps has employed the Dragendorff test[13, 14] as one of the methods for differentiating between the sulfur and nitrogen mustards. The nitrogen mustards react with an acidic solution of bismuth iodide complex to form an insoluble, bright orange-red precipitate. The vapors are collected on unimpregnated silica gel. After the addition of Dragendorff's reagent, the appearance of an orange-red color on a yellow background indicates presence of nitrogen mustard. Buswell[15] has recommended this method for the detection of nitrogen mustard in water also.

Cruikshank et al[16] proposed several different chemically treated papers for the detection of bis-(2-chloroethyl) methylamine in both

liquid and vapor states. The vapor detector paper is a modification of Dragendorff's method, but by the addition of calcium chloride hexahydrate and glycerol, the stability of the paper was increased. A positive reaction of a bright red color could be obtained by 25 micrograms of bis-(2-chloroethyl) methylamine. By adding tartaric acid to the reagent, traces of bis-(2-chloroethyl) methylamine can be detected in water. Paper treated with cobalt-titanium salts and sodium thiocyanate gave a blue color reaction for bis-(2-chloroethyl) methyamine. Nickel dimethylgloxine is very sensitive to the nitrogen mustards, but it is not specific because the red color is also produced by gases which are appreciably basic in solution. The sulfur mustard and chloroarsenicals failed to produce any color reaction with these three treated papers.

DB-3 Test for Mustard Gases

The Chemical Corps developed the most satisfactory reagent for detection of sulfur and nitrogen mustards. It is more commonly referred to as the DB-3 reagent than by its chemical name of gamma-(4-nitrobenzyl) pyridine. Recently, Epstein[17] published some of the uses of this reagent for the determination of ethylenimines and alkylating compounds. It has been chiefly used for its reaction with the mustard gases at elevated temperatures to form a colorless addition product which reacts with strong alkalies to form an intense purple-blue dye.



Several different methods of utilizing the DB-3 reaction to detect mustards in air, water, and on surfaces have been developed by the Chemical Corps. For the detection in air, the reagent is impregnated

in silica gel and placed in a small glass tube. Suspected air is drawn through the tube by means of a small hand pump or rubber bulb, and the agent is absorbed on the silica gel. Heat needed for the reaction can be supplied by the flame from a match or a chemical heater. The chemical heater incorporated with the test in Army's "Chemical Agent Detector Kit, A2M9" consists of an aluminum foil sleeve surrounding the tube which, when treated with copper chloride solution, generates heat.

$$3CuCl_2 + 2\,Al \longrightarrow 2\,AlCl_3 + 3\,Cu + Heat$$

After this reaction has ceased, a few drops of sodium hydroxide solution are added to the silica gel. The formation of a purple-blue color in the tube indicates the presence of sulfur or nitrogen mustard in the air sample. About 0.001 milligram per liter of air can be detected readily. Semiquantitative results can be obtained by correlating the amount of air drawn through the tube and the amount of color produced.

About the same principle is employed for the detection of mustards on surfaces or on foods. The silica gel, containing DB-3 reagent, is adherent on one side of a small strip of paper, and the aluminum foil on the other side. The side containing the silica gel is pressed against the suspected surface to absorb the agent. Heat is produced by addition of copper chloride reagent to the aluminum foil on the reverse side and then the addition of sodium hydroxide reagent to the silica gel. The appearance of a purple-blue spot indicates a positive test.

For detection in water, the sample is first adjusted to approximately pH 4. A small aliquot is then placed in a test tube; 0.5 ml of 5% DB-3 solution in acetone is added, heated, and made alkaline. Positive test is indicated by purple-blue coloration.

This DB-3 test responds to many chemical compounds that have the properties of alkyls and act as strong alkalting agents. Epstein[17] lists numerous compounds that respond to this test, however, most of them would not be encountered when examining air, water, or food for C. W. contamination. Some other C. W. agents or reactive compounds which might be encountered and produce color with this test are:

Agent	Color reaction
Sulfur mustard	purple-blue
Nitrogen mustards	purple-blue
Chloropicrin	brown
Chloroacetonephenone	reddish-violet
Ethyl bromoacetate	blue
Dimethyl sulfate	blue
Methyl iodide	blue
Benzyl chloride	reddish-violet
Xylyl bromide	blue
Sulfur chloride	brown

In addition to responding to alkyl compounds, DB-3 reacts with cyanogen chloride, cyanogen bromide, and bromobenzylcyanide to form a yellow or orange color immediately or when heated without the need of alkali.

Gamma-(4-nitrobenzyl) pyridine is not available commercially. It is specially prepared by the Chemical Corps for use in all the kits. It can be synthesized in the following manner[18]: Dissolve 5.0 g of gamma-benzyl pyridine in a small volume of concentrated sulfuric acid. While cooling and stirring constantly, slowly add 3.5 g of concentrated nitric acid and 15 g of concentrated sulfuric acid. Let stand overnight and then pour into about 500 ml of distilled water. Extract the aqueous solution with several quantities of ethyl ether. Combine the extracts, dry, and distill off the ether. An oil remains which crystallizes on standing. Recrystallize from alcohol to obtain prismatic crystals, m.p. 74°C.

ARSENICAL C.W. AGENTS

In the earlier stage of chemical warfare a large number of organic arsenical compounds were evaluated. Many of these are no longer considered for used because of the newer agents that supersede them in requisites for chemical warfare agents. Those that remain are:

Name	Formula	Symbol
Beta-chlorovinyldichloroarsine	$ClCH{:}CHAsCl_2$	L
Phenyldichloroarsine	$C_6H_5AsCl_2$	PD
Ethyldichloroarsine	$C_2H_5AsCl_2$	ED
Methyldichloroarsine	CH_3AsCl_2	MD

Studies by Green and Price[19] on lewisite (beta-chlorovinyldichloroarsine) showed that with dilute solutions of sodium hydroxide lewisite decomposed to arsenious acid and acetylene.

$$Cl\text{-}CH = CH\text{-}AsCl_2 + 6\ NaOH \longrightarrow C_2H_2 + Na_3AsO_3 + 3\ H_2O$$

The fact that this reaction was specific for lewisite offered a method for distinguishing lewisite from other arsenical compounds. Lewis and Perkins[20] used this reaction for the quantitative determination of lewisite by measuring the volume of acetylene liberated. Later, Ilosvay[21] colorimetric test for acetylene was employed. Acetylene produces a red to brown color of cuperous acetylide when passed through an ammonical solution of cupric nitrate or the chloride, iodide or sulfate salt of copper. Many modifications of this method were developed. The test was simplified by impregnating the cupric nitrate reagent in paper for testing acetylene after the air is passed through a 15% solution of sodium hydroxide [22, 23, 24]. Kouten, Shohon and Munn[25] used the same principle in a design of a field apparatus for vegetation and soil contaminated with lewisite. One of the Army's [26] current procedures is to aspirate the air through silica gel impregnated with cuperous iodide and then moisten the silica gel with 10% sodium hydroxide solution. The Army's test is sensitive to as little as 3 micrograms of lewisite.

Sartori[27] describes a method for differentiating lewisite from the other alkyl dichloro-arsines. It is based on the reaction of the arsines with a solution of mercurous nitrate acidified with nitric acid. With lewisite a white precipitate is formed; with ethyldichloroarsine a white precipitate is formed, which slowly turns gray within a few minutes; methydichloroarsine produces an immediate gray precipitate of metallic mercury.

Branger and Mercier[28] noted that chloroform solutions of ergosterol and methydichloroarsine would produce a golden yellow color when mixed together. The density of the color was proportional to the concentration of methyldichloroarsine. Mason[29] investigated the same reagent as an indicator for beta-chlorovinyldichloroarsine in the air. Silica gel was impregnated with ergosterol from a chloroform solution and dried at room temperature. On contact with traces of beta-chlorovinyldichloroarsine vapors, an immediate violet color resulted, changing to green in the presence of larger amounts. The test, however, also produces similar but a fainter color on long exposure to air saturated with sulfur mustard vapors.

Recently Yoe and Coghill[30] investigated the color reaction of 15 organic reagents with beta-chlorovinyldichloroarsine and ethyldichloroarsine. All the reagents produced some color reaction with lewisite and/or ethyldichloroarsine when the reagents were impregnated in paper and exposed to the liquid state of the agent and treated with sodium hydroxide. Sulfur and nitrogen mustards were used as controls and failed to produce color with any of the 15 reagents. Of the reagents reported, m-dinitrobenzoyleneurea was considered as the best indicator for lewisite. It produced a red color. An investigation was also made for a vapor test for lewisite using p, p-dinitrostilbene-o, o-disodium sulfate. An absorbent was impregnated with the reagent and packed in a small glass tube. Arsenical vapors were drawn through the tube and treated with one ml of sodium hydroxide solution. A detectable color was produced by 25 micrograms of lewisite and 3 micrograms of ethyldichloroarsine.

The detection and quantitative analysis of the arsenic ion is considered to be the best means of testing for arsenical warfare agents as a group. Earlier methods of titrating the oxidized arsenical compounds iodometrically lack sensitivity and are considered inferior to colorimetric methods of molybdenum blue and mercuric chloride.

Ruchhoft, Placak, and Shott[31] made a comparative study of several methods for the determination of arsenic in water contaminated with chemical warfare agents. Three preliminary treatments were studied for their ability to quantitatively oxidize organic arsenical agents so that the arsenic could be determined by the molybdenum blue or mercuric bromide (Gutzeit) methods. Oxidation with chlorine to satisfy a 5 to 10 minute chlorine demand or cold permanganate yielded less than 50% recovery of the arsenic. Essentially complete recovery of arsenic was obtained from lewisite, adamsite, and diphenylchloroarsine by the hot acid permanganate oxidation, but not for ethyldichloroarsine and methyldichloroarsine. For the complete oxidation of all the arsenical warfare agents in water, wet digestion with concentrated sulfuric and nitric acid is required. The two methods for the determination of arsenic were also evaluated. The Gutzeit method, using mercuric bromide paper strips was considered time consuming due to all the precautions needed and could not be relied upon for accurate quantitative results by the average analyst. It could only be considered as a semiquantitative method. The molybedenum blue procedure is affected by silicon and phosphate

interferences when applied directly to the digested sample, however, they can be eliminated by distillation of the digested sample with hydrogen and collecting the arsine in a hypobromite trap. This is now the standard method[32] for the determination of total arsenic content of water.

Recently, Berkhout and Jangen[33] modified the method of collecting the arsine gas by using a cellulose pad treated with mercuric bromide. The author claims this method of absorption to be more efficient than a sodium hypobromite solution, an acidic solution of mercuric bromide containing permanganate or sodium bicarbonate solution of iodine. The absorbent pad is then washed with sodium hypobromite and determined by the molybdenum blue method.

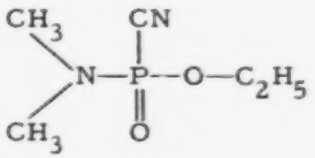
Adamovick and Rybuikova[34] removed arsenic from water by boiling with ferric alum at a high pH. The ferric hydroxide precipitate containing the absorbed arsenic is filtered, dissolved in dilute hydrochloric acid determined colorimetrically with sodium hypophosite (Bougault's reagent)[27]. The method is sensitive to 0.02 ppm arsenic. Earlier work reviewed by Mellor[35] showed the removal of arsenic from water with ferric alum was influenced by pH, temperature, and the ratio of the amount of arsenic and alum present. Buswell[36] made a similar study on the removal of arsenic by coagulation for the purpose of purification in water plants. Laboratory jar test indicate some reduction of arsenic is obtained at room temperature and a pH between 6 and 7.

For the detection of alkylchloroarsines in air, Wilzback[37] developed a chemical indicator that is very sensitive which can be used as a field test. The principle is based on the molybdenum blue reaction. A silica gel impregnated with 1% zinc sulfate and 5.5% molybdic acid will produce a blue to green coloration with 1 microgram of ethyldichloroarsine, 2.5 micrograms of methyldichloroarsine or 3 micrograms of phenyldichloroarsine. The indicator is not particularly sensitive to chlorovinyldichloroarsine especially under conditions of high humidity but the sensitivity can be improved by heating to 100°C for one minute. The test is negative for arsine and large quantities of all the other chemical agents. The Army[38] incorporates a similar method for the detection of organic arsenicals in their air sampling kit. For an immediate reaction, the addition of sodium hydroxide solution to the contaminated indicator is suggested.

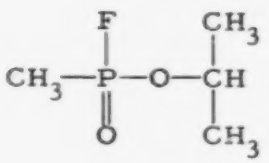
G-AGENTS AND RELATED COMPOUNDS

At the end of World War II a group of highly toxic organic phosphorus compounds intended to be used as chemical warfare agents were found in the possession of the Germans. Such compounds are the most dangerous chemical agents known today; they act upon the body by destroying the nerve regulatory enzyme cholinesterase. Although these agents are highly volatile liquids, they are called nerve gases or the G-series of CW agents. The structural formula for three of the most important G-agents are:

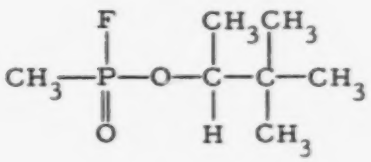
Tabun



Sarin

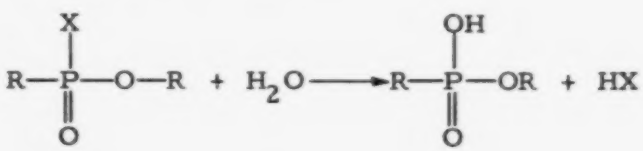


Soman



No chemical methods for the detection of these agents have been published in open literature. However, by visual examination of the structure of these compounds, several nonspecific methods may seem likely, such as the detection of the cyanide, or fluoride radicals.

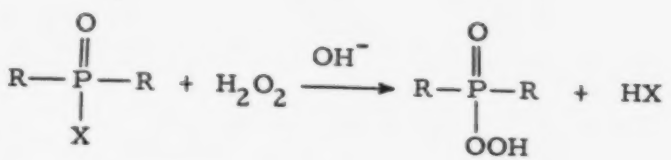
These compounds hydrolyze in water at variable rates, depending upon the pH, temperature or quantity of dissolved solids to hydrogen fluoride or hydrogen cyanide.

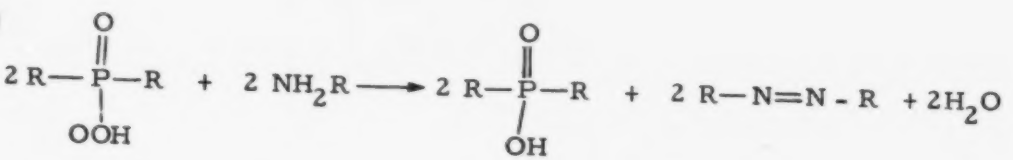


In employing the fluoride or cyanide radical for the detection, the sensitivity would be limited to sensitivity of the fluoride or cyanide test. In drinking water the toxic limit for tabun is about 2-3 ppm. When it is completely hydrolyzed at this concentration, it is equivalent to 0.32 to 0.48 ppm cyanide. This concentration of cyanide can be determined by the pyridine-pyrazolone method[40, 41]. Sarin and soman toxicity limit in water is only 1 ppm. Complete hydrolysis of a 1 ppm of sarin or soman would yield 0.10 or 0.14 ppm of fluoride respectively. Although this low quantity could be determined by standard methods[42] the results would not be significant since the natural fluoride content may be several times this amount.

A colorimetric chemical test[43] was developed by the Army Chemical Corps shortly after World War II for the detection and determination of G-Agents. This test is now included in field kits, "Civil Defense Kit, Water Testing and Screening", No. CD V-902 (same as Army number AN-M-2), "Civil Defense Kit, Water Testing, Poisons", No. CD V-907 (same as Army number AN-M4) and "Civil Defense Kit, Food Testing and Screening", No. CD V-903 (same as Army number ABC-M3) for the detection of CW agents in air, water, and on food or surfaces.

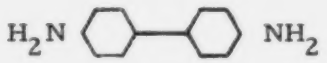
This test for G-agents is based on the Schoenemann reaction which results in the formation of azo-type dyes. In the presence of alkaline-peroxide solutions, compounds containing a labile-metalloid halogen linkage form an intermediate product of strong oxidizing power that oxidizes amino bases to produce a color reaction. With organo-phosphorus compounds the mechanism of this reaction was expressed by early investigators by the following equations:

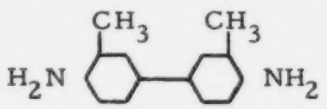
(1) 

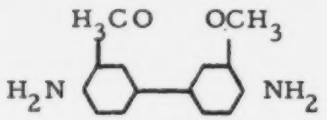
(2) 

X = CN^-, F^-, Cl^-, or Br^-

This reaction is specific for organic phosphorus compounds that can be oxidized by alkaline peroxides to the intermediate phosphorus peroxide. Several organo-phosphorus insecticides such as tetraethyl pyrophosphate (TEPP) and hexaethyl tetraphosphate (HETP) not belonging to the G-series also produce a positive color. The amino bases investigated for this reaction were:

benzidine 

o-tolidine 

o-dianisidine 

Satisfactory color formation was obtained by each; ortho-dianisidine, however, produced a slightly greater density of color.

The method is also adaptable for the quantitative determination of G-agents in water. The color formation conforms to Beer-Lambert's Law and is affected very little by temperature or constituents normally found in water. The presence of reducing agents interfers with the production of color. When the chlorine demand of water is greater than 10 ppm, it is recommended that the demand be reduced to about 1 ppm in order to eliminate such interferences.

Many of the new organo-phosphorus insecticides are related to the G-agents by their chemical structures and physiological properties. Methods for their detection may be applicable to the G-agents. It is the opinion of Kodama et al[44], after an extensive review of the toxicity of organo-phosphorus compounds, that their toxicity is primarily dependent on the degree of chloinesterase inhibition that they exert. Many methods[45] have been studied for the measurement of cholinesterase activity of blood and plasma effected by these insecticides. Only recently, however, has the determination of the insecticides been investigated by inhibiting the cholinesterase activity "in vitro".

Cholinesterase hydrolyzes acetylcholine to acetic acid and choline. When an inhibitor is present a portion of the enzyme is inactivated, thus reducing the amount of acetylcholine hydrolyzed. The reactions

involved are:

(1) Cholinesterase + inhibitor ⟶ inhibited cholinesterase + uninhibited cholinesterase.

(2) Uninhibited cholinesterase + acetylcholine ⟶ acetic acid + choline

The quantity of acetylcholine not hydrolyzed is proportional to the inhibited enzyme and therefore is proportional to the inhibitor. The pH change caused by the liberation of acetic acid has been measured by alkaline titration, pH indicators[48], and electrometric methods[47, 49]. Early investigators regarded a manometric method of Ammon[50] as the most accurate means of determining the acetic acid. Carbon dioxide is liberated from the reaction of acetic acid with a bicarbonate solution. Fleisher[51] determined the cholinesterase activity by a colorimetric method of measuring the residual acetylcholine. This colorimetric method has the advantage of a more rapid analysis, relative simplicity of operation and greater specificity than the previous methods. About the same time Cook[46] proposed a similar colorimetric method for the determination of organic phosphorus insecticides by cholinesterase inhibition based on the same reaction. The Food and Drug Administration[52] is currently making a collaborative study of this method for determining the amount of residual organic phosphorus and carbamate insecticides on fruits and vegetables. Although this method would be too sensitive for the detection of G-agents in water at the usual toxic level, satisfactory results might be obtained by serial dilution of the samples. The advantages of a cholinesterase test is that it would be applicable for the detection of all compounds whose toxicity is primarily dependent on its cholinesterase inhibiting power.

OTHER TOXIC CHEMICAL COMPOUNDS

In some of the early investigations of the fluoro-organic compounds having a carbon to fluoride bond, the fluoro-acetates and fluoroethanols were proposed as toxic water contaminants. They were recognized as exceedingly dangerous if present in water supplies because of their stability and their lack of taste and odor[53]. Methods of detecting them depend on the cleavage of the fluorine and the identification of one or both fragments of the molecule. However, the detection of fluoride as a means of indicating contamination is limited to these compounds where at least 50 ppm are needed to produce a harassing dose[54]. The fluorine atom is remarkably inert; therefore, vigorous oxidizing methods are needed to separate its linkage to the carbon atom.

At the Chemical Corps laboratories two field methods[54, 55] were developed for the detection of fluoro-organic compounds in water. The fluoride cleavage was attained by burning the suspected water in a special alcohol burner. The fluoride vapors are then collected in a small quantity of distilled water and determined colorimetrically by its bleaching effect on a Solochrome Brilliant Blue-thorium lake. The other method is known as the "spot test". The fluoro-organic compound is oxidized by potassium persulfate and evaporated to dryness. The residue is checked for the fluoride content by the addition of a buffered Chrome Azurol-thorium nitrate indicator. Any reddening, as compared with the blank, is considered a positive test for at least 5 ppm fluoride.

Detection of Organic Fluoride Compounds
in Edgewood Tap Water by the Spot Test Method

Organic fluoride compounds			
Agent	Conc. ppm	Equivalent F concentration ppm	Results
Fluoroethanol	33.4	10	positive
Methyl fluoroacetate	24.3	5	positive
Sodium fluoroacetate	26.3	5	positive
Diisopropyl fluorophosphate	48.5	5	positive
Beta fluoroethyl omega fluorobutyrate	20.0	5	positive
Blank		None	negative

The British reported a specific test for sodium fluoroacetate known as the chromotropic acid test[56]. Alkaline solutions containing fluoroacetates were evaporated to dryness and heated to effect cleavage of the agent to sodium glycollate. Addition of

sulfuric acid decomposed the glycollate to formaldehyde, which was detected by the intense purple color it produced when treated with chromotropic acid (1, 8-dihydroxy-3, 6-naphthalene disulfonic acid). In conjunction with the fluoride test, a fairly specific detection of fluoroacetate down to one part per million is possible.

Price[56-60] stated that "vigorous oxidation of fluoroacetates with sulfuric or chromic acid causes complete cleavage to carbon dioxide, hydrogen fluorode and water." This statement indicates that the distillation procedure of fluorides, as in Standard Methods[57], would also cause the cleavage of the fluoride. If this is true, then the standard method for fluoride determination could serve as a quantitative laboratory method for fluoro-organic compounds in water.

CHLORINE DEMAND

Most of the war gases or their hydrolyzed products when present in water will react with chlorine and may be detected by routine chlorine demand procedures. The residual chlorine content of all treated water is generally the last analysis to be made on the water before consumption. If the water contains no residual chlorine, it may be the first objective indication of any type of contamination. For this reason the chlorine demand is considered a most valuable method for the detection of many of the chemical warfare agents in water.

The chlorine demand of water is the difference between the amount of available chlorine applied and the amount of the residual chlorine remaining at the end of the contact period. The demand for a given water will vary with the amount of chlorine applied, time of contact, pH, and temperature. Chlorine demand test, when applied to the detection of CW agents, is generally determined between pH 1 and 2 with at least 7 ppm of applied chlorine after a contact period of five minutes at approximately room temperature.

When water, containing sulfur mustards or their hydrolyzed products, is chlorinated the following reactions occur:

$$(ClCH_2CH_2)_2S + CL_2 + H_2O \underset{OH^-}{\overset{H+}{\rightleftharpoons}} (ClCH_2CH_2)_2SO + 2\ HCl$$

sulfoxide of mustard

$$(ClCH_2CH_2)_2SO + Cl_2 + H_2O \underset{[OH^-]}{\overset{[H^+]}{\rightleftharpoons}} (ClCH_2CH_2)_2SO_2 + 2\,HCl$$

sulfone of mustard

With mustard gas in tap water, Buswell et al[36] found that for a 5-minute reaction time and at pH 1.0, the chlorine demand equaled the amount (ppm) of sulfur mustard added. Between pH 6 and 7 the demand equaled half of the amount added, and above pH 10.5 no demand was exerted. This was also true of its hydrolyzed product thiodiglycol. With lewisite the demand was equimolar over the entire pH range.

The three nitrogen mustards exhibit a chlorine demand with sodium or calcium hypochlorite only at pH 8[58, 59].

$$(ClCH_2CH_2)_2NR \xrightarrow[pH\ 8]{O\,Cl^-} (ClCH_2CH_2)_2NCl$$

$$R = CH_3,\ C_2H_5 \text{ or } CH_2CH_2Cl$$

Although it is possible under controlled conditions to use the chlorine demand test as a quantitative measure[36, 10] for some CW agents, it is generally considered as a means of detection. Chlorine demand test, as in the Civil Defense Kit, Water, Testing and Screening, No. CD V-902, is applied to the detection of sulfur mustard, lewisite, methyldechloroarsine, ethyldichloroarsine, diphenylcyanoarsine, trivalent arsenic as arsenite and cyanides. A positive test for at least 3 ppm of the agents is indicated by failure to obtain a residual chlorine of 1 ppm or more after 5 minute contact period when using an initial dose of 6 ppm chlorine.

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